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REDISTRIBUTION OF BLOOD VOLUME DURING THE ONSET OF DEOXYCORTICOSTERONE ACETATE-SALT HYPERTENSION

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REDISTRIBUTION OF BLOOD VOLUME DURING THE ONSET OF DEOXYCORTICOSTERONE ACETATE-SALT HYPERTENSION

By

Bridget Mahon Seitz

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ABSTRACT

REDISTRIBUTION OF BLOOD VOLUME DURING THE ONSET OF DEOXYCORTICOSTERONE ACETATE-SALT HYPERTENSION

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The splanchnic veins are known to hold the largest amount of blood within the circulation. Structural and/or neurohormonally mediated changes in the diameter of these vessels can lead to a reduction in splanchnic vascular capacitance and blood volume. This causes an increase in cardiac filling pressure as blood is translocated towards the heart. The resulting redistribution of blood into the arterial circulation could be a factor in the development hypertension. Previous data suggests that a reduction in vascular capacitance may play a pivotal role in the pathogenesis of hypertension in the DOCA-salt model, but no direct measurement of volume shifts have been reported. The purpose of my research is to assess blood volume redistribution during the onset of DOCA-salt hypertension using bioimpedance measurement. Regional bioimpedance allows for the measurement of total fluid content in specific body compartments. For this study I developed a method for repeated measurements of regional bioimpedance in conscious, unrestrained rats over several weeks. Using this technique, I am able to show an increase in impedance in the abdominal region in DOCA-salt treated rats (most likely indicating a decrease in fluid content). I conclude that fluid translocation from the abdominal region due to decreased venous capacitance may participate in the development of DOCA-salt hypertension

Dedication

To my Mom, thank you for all your loving support and wonderful weekly visits. You will never know how much it meant to me.

To my dear, Dad, who never was able to see the completion of this degree. I finished for you!

To my wonderful children: Mitchell, Natalie and Cameron, thank you for your many hugs and best of all your laughter. Being your mom will always be my best job.

To my best friend and husband, Ted, thanks for always being my rock. Your continual patience and confidence in me made this possible. I could never have done it without you!

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Introduction

1. Hypertension

Hypertension is defined as a sustained elevation in systemic arterial pressure with a systolic blood pressure >140mmHg and/or a diastolic blood pressure of >90mmHg.[1] The World Health Organization estimates that nearly 1 billion people have hypertension worldwide. This number is predicted to increase to 1.5 billion by 2025.[2] The global disease burden attributable to hypertension is substantial because hypertension plays a major etiologic role in the development of other cardiovascular conditions.[1-3] It has been shown that even small increments in blood pressure are associated with increased target-organ morbid outcomes, such as stroke, coronary artery disease, renal disease and peripheral vascular disease.[3] As a result, the threshold values for defining hypertension are ever evolving. In fact, the Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure has introduced a new category, "pre-hypertension," to describe individuals with blood pressure values as low as 120/80 mmHg.[1-3] Most importantly, pre-hypertension is frequently considered a "precursor" to hypertension.[4] Alterations in cardiovascular structure and function have been shown in recent studies to precede the finding of elevated blood pressure.[4] These findings have included the occurrence of left ventricular hypertrophy,[4-6] diastolic filling abnormality,[6] endothelial dysfunction,[7] as well as impairment in autonomic regulation[8] as precursors to hypertension. Whether pre-hypertension alone, or association with its common cardiovascular risk factors, leads to hypertension is still uncertain. However, these results provide evidence

that mechanisms operating during the early development of hypertension could be crucial in producing the stable form of the disease.

While there is no cure for high blood pressure, it is controllable by many different behavioral and pharmacological interventions.[1] Thus, managing high blood pressure is a lifetime commitment, adding greatly to its economic impact on society. Further insight into the pathophysiological processes that occur during early hypertension development may provide treatment strategies to delay or prevent the full-blown disease and attendant target organ injury and the associated economic burdens on society.

A single main cause for hypertension is discernable in only 5-10% of human patients.[1] Examples of such causes are renal artery stenosis, renal parenchymal disease, and mineralocorticoid secreting tumors.[9] The remaining 90-95% of hypertensive individuals are said to have "primary" or "essential" hypertension.[1] Genetic as well as behavioral factors contribute to essential hypertension,[10] but to widely differing degrees in any one individual. Thus, there are a myriad of "causes" for essential hypertension, however all ultimately lead to a defect in the regulation of blood pressure. Likely causes of high arterial pressure in essential hypertension include: over activity of the sympathetic or renin-angiotensin-aldosterone systems; renal dysfunction leading to excessive retention of sodium and water; impaired endothelial cell release of vasodilators; and structural changes in large and small arteries associated with aging.[12]

2. Overview of the Circulatory System

The regulation of arterial pressure involves many complex processes that govern total circulating blood volume, and the function of the heart and vasculature. To aide in understanding the potential causes of increased pressure within the arterial system, a brief overview of the circulatory system is necessary.

The peripheral circulation (excluding the pulmonary circulation) is a semi-closed, blood-filled circuit that consists of a high-pressure arterial side and a low-pressure venous side arranged in series. Capillaries connect the two sides where oxygen, nutrient and metabolite exchange occurs between blood and tissue. The venous and arterial circulations work in balance and are controlled by neuronal and humoral factors that help achieve overall homeostasis of the circulatory system.[12-13] The main function of the arterial vessels are to distribute oxygenated blood from the heart to peripheral organs such as the muscle, viscera and brain. In addition, small arteries and arterioles in various organs within the circuit control regional distribution of blood flow. This distribution by adjusting the arterial lumen diameter through changes in the tone of the vascular smooth muscle located within the walls of this vessels.[12] In contrast, the main function of the venous system is to collect deoxygenated blood and return it to the heart. Some veins also serve as important blood holding (capacitance) vessels. Veins are 30 times more compliant than arteries and therefore are capable of holding relatively large blood volumes without alterations in their intraluminal pressure.[13] In conclusion; arterial function

regulates resistance and flow through the circulation, while venous function regulates capacitance and volume.[14]

Arterial blood pressure is typically described (using an electrical analogy, i.e. Ohm's Law) as the product of two hemodynamic variables, one directly measured and the other calculated. Cardiac output (CO) can be directly measured as the volume of blood that is ejected from the heart and flows through the arterial system per unit time; it is the product of heart rate (HR) and stroke volume.[12] Systemic vascular resistance (SVR) is a calculated variable that reflects the many forces within the circulatory system that impede blood flow; the most important of these forces is the vascular diameter of small arteries and arterioles.[15] Hypertension occurs when the value of one (or both) of these hemodynamic variables (CO and SVR) is increased above the "normal" range for arterial pressure.

In general, the onset of essential hypertension in humans has been shown to be associated with an increase in CO, whereas in the established phase of hypertension SVR is increased in most people.[16-17] Hence, the majority of research with regards to the causes of hypertension have focused on mechanisms that affect either the diameter of small arteries and arterioles, or systemic blood flow driven by the heart (primarily total circulating blood volume or cardiac function *per se*. However, arterial pressure also can be simply expressed as the blood volume within the arterial system divided by the *compliance* of the arterial system[18] Compliance reflects the ability of a vessel to distend and increase volume with increasing

transmural pressure (i.e. the difference between intraluminal and extramural pressure).[13] This understanding of arterial pressure regulation does not focus on the steady-state *flow* of blood through the arterial system (CO), but instead on steady-state changes in arterial *volume* which can be brought about by transient *differences* in arterial system inflow (determined by CO) and outflow (determined by vascular resistance). These volume differences are well-understood to account for the pulsatile nature of arterial pressure created by cyclic cardiac contraction and relaxation, but are less often considered explicitly as a major determinant of the chronic level of arterial pressure (which is key to the diagnosis of hypertension). This is probably due to the fact that flow through the circulatory system can be readily measured, whereas the volume of the arterial system is difficult to determine accurately. Nevertheless, within this conceptual framework, possible mechanisms of hypertension are either: 1) a *decrease in arterial system compliance,* or 2) an *increase in steady-state arterial system volume.* Decreased compliance of the aorta and large arteries is widely believed to account for the specifically elevated systolic pressure (isolated systolic hypertension) that is often observed with aging, and in patients with established essential hypertension.[19] Small artery compliance is often normal, however can even be increased in essential hypertension, or decreased with age or in cases of severe hypertension.[19] However, the focus of the research described in this thesis is on how changes in *vascular volume* may affect arterial pressure.

3. Blood volume distribution in the circulation

Blood is held within all parts of the cardiovascular system, with approximately 70% of blood volume is contained in the venous system.[13] "Active capacitance" veins are filled or emptied by changes in tone of the smooth muscle within their wall.[20] "Conduit" veins are mainly filled and emptied by passive forces associated with gravity or external compression by surrounding tissue.[13] Therefore, either changes in venous vascular tone (venoconstriction) or passive changes in venous diameter, can cause dramatic shifts in blood volume within the circulation.

There are two compartments within the venous system: central and peripheral. Importantly, veins within these two compartments are not uniform in their compliance or capacitance.[13,21] Veins in the peripheral venous compartment are very compliant and have a high capacitance.[14] The splanchnic veins, located in the abdominal region, represent the largest blood volume reservoir within the human body[13], and exhibit the greatest degree of active capacitance response of any veins in the body.[20] Veins in the central venous compartment are far less compliant.[14] The thoracic vena cava and the great veins of the central venous compartment are unable to store blood volume to any great extent.[13] However, it is the central veins that are critical for circulatory dynamics.[21] Any stimulus that causes a decrease in peripheral venous compartment capacitance (active or passive) will redistribute blood from the peripheral to the central venous compartment. Because of the low compliance of the central venous compartment, this augments the amount of blood entering the heart (venous return). This increased volume of blood entering the

heart transiently exceeds the amount of blood leaving the heart (cardiac output), resulting in enhanced cardiac filling pressure and volume.[14] This leads, via the Frank-Starling mechanism, (increase in pre-load results in an increase in contractility), to augmented blood ejection by the left ventricle, and a portion of the excess blood that entered the heart being redistributed into the systemic circulation, including the arteries.

Changes in venous vascular capacitance can also function as an important physiological compensatory mechanism, adjusting the circulation in response to everyday stresses. The hemodynamic response to exercise illustrates the dynamic changes within the venous vasculature required to respond to the requirements of the body for blood and oxygen delivery and waste removal. During exercise, as the artery supplies blood and oxygen to the working muscle, veins undergo both active and passive emptying, i.e. there is a decrease in venous vascular capacitance and a shift of "stored" blood towards the central circulation, increasing venous return (VR, flow of blood to the heart), CO and arterial pressure.[22] Similar adaptations can also be seen under normal physiological conditions even without physical exertion. For example, performing a challenging arithmetic problem can alter venous capacitance. In one study, subjects performing difficult subtraction problems had an increase in forearm venoconstriction, which caused blood to be displaced towards the heart, increasing CO and arterial pressure.[23] Thus, venous vascular capacitance is important in the regulation of arterial blood pressure in a normal physiological state as well as, in a pathological state, such as hypertension.

4. Blood Volume and Arterial Pressure Regulation

Despite many years of research, there is no unifying theory to account for the pathogenesis of essential hypertension. What has been shown is that during the early stage of arterial pressure elevation there is often an increase in central blood volume and transient increases in stroke volume and CO.[15-17] Eventually, as the disease becomes more long- standing, these hemodynamic processes revert to physiologically normal and hypertension is continually sustained by an elevation in systemic vascular resistance.[12,15]

According to this scenario, during the onset of hypertension blood volume increases in the central venous compartment, cardiac filling rises, and the Frank-Starling mechanism leads to more blood volume being expelled into the low compliant arterial beds. This results in an elevation in CO, arterial volume and arterial pressure.[21] It is important to note that under normal physiological condition, the heart and lung have relatively low and fixed blood storage capacities. Thus, in this discussion, the pulmonary circulation is not a factor and the heart is represented as merely a "demand" pump, which moves blood from the low pressure, high compliant venous system into the high pressure, low compliant arterial system.

In accordance with this model, two general mechanisms could produce an increase in central blood volume and contribute to a rise in arterial pressure. The first mechanism is an increase in the total volume of blood contained within the entire circulation. When total circulating blood volume increases, blood volume and/or

blood pressure increases in all vascular compartments in relation to their compliance. [20]

Total blood volume is a relatively fixed fraction of the extracellular fluid volume and is determined primarily by the balance between sodium (the main extracellular osmotic particle) and water intake, and renal sodium and water excretion.[24-25] The major site of regulation of sodium and water homeostasis is the kidneys. Therefore, renal function has a major influence on total circulating volume, and as a result, arterial pressure.[12,24-25] In fact, arterial blood pressure has often been used as a key indicator of the volume of extracellular fluid within the circulation.[26]

Although that principle is well accepted, in the 1960's Arthur Guyton and his colleagues proposed an additional relationship between arterial pressure and renal function that has been more controversial. Their proposition was that renal sodium and water excretion are directly proportional to the prevailing level of arterial blood pressure (the so-called "pressure-natriuresis relationship").[27-29] The implication of this dual cause-and-effect relationship between arterial pressure and renal function are elaborated in great detail in numerous papers and their now famous mathematical model of the circulation.[28] TheGuyton mathematical model affirms that the level of steady-state arterial pressure is determined *entirely* by the ability of the kidney to excrete sodium and water. [28,30] According to this hypothesis, hypertension can *only* occur when the pressure natriuresis relationship is altered in a way that leads to the kidneys retaining more sodium and water at a given

pressure, thereby producing at least a transient increase in total blood volume in the circulation.[26-31]

An increase in total blood volume alone has been shown to elevate arterial pressure and contribute to the development of hypertension.[32-34] This is evident in some obese borderline hypertensive patients, which have increased total blood volume compared to age-matched lean borderline hypertensive patients. In addition, 90% of the cases of hypertension in patients undergoing hemodialysis for end stage renal disease are specifically due to sodium and volume overload.[36] In support of these findings, adequate reduction and control of total blood volume achieved normotension in these previously hypertensive hemodialysis patients.[37]

These examples represent specific instances where total blood volume parallels arterial pressure. However, this is not always the case. The literature most often reports total blood volume to be reduced in essential hypertension and inversely associated with arterial pressure.[15-16,20-21,38-41] This inverse relationship is further supported by situations where higher than normal total blood volume occurs while blood pressure is below normal, such as in hepatic cirrhosis[42] and pure autonomic failure.[43] In conclusion, total blood volume can, but is not always consistent with arterial pressure.

The focus of my research is on a second possible mechanism to produce increased arterial blood volume and thereby arterial hypertension: a *redistribution* of the total

blood volume within the circulation. With this mechanism, no increase in total blood volume or impairment in renal function, is required; in fact it can operate in the presence of a reduction of total blood volume (as is often observed in individuals with hypertension).[15,38-40] Instead of arterial blood volume being elevated due to an overall increase in body fluid volumes, this mechanism consists of a translocation of blood from the highly compliant, blood storing venous circulation into the low compliant, high pressure, arterial system. In support of this idea, earlier research demonstrated that during the early phase of hypertension development there was a higher fraction of the total circulating intravascular volume in the central circulation.[16] This was found without any alterations in total blood volume. Theoretically this redistribution of blood volume could be achieved by any mechanism (for example, vigorous muscular exercise, as discussed above) that decreases overall venous capacitance of the peripheral venous compartment, and leads to the mobilization of blood volume into the central venous compartment and then into the arterial system.

The two mechanisms described above that produce elevated arterial system volume and pressure are not *mutually exclusive*, nor are they *exhaustive*. Another means to increase arterial volume is via increased resistance in small arteries and arterioles, which effectively "traps" an increased volume of blood in the upstream arterial tree.[12] This mechanism is most often observed in established forms of hypertension.[16-17] All three of these mechanisms operate together under normal physiological circumstances, and all potentially contribute to the pathophysiology of

hypertension. However, the relative impact of volume redistribution on arterial pressure regulation is often underestimated as a significant contributor.

An example of the significant influence of blood volume redistribution on arterial pressure can be observed in *supine hypertension* in individuals with peripheral autonomic failure.[43] While most of these patients have abnormally low arterial blood pressure—due to a decrease in blood volume or an increase in peripheral venous pooling (from gravitational force) when standing—dramatic hypertension occurs in these patients when supine.[44] The likely explanation for the occurrence of hypertension while patients are in the supine position is a redistribution of blood volume from the highly compliant splanchnic veins towards the central circulation and ultimately into the arterial system (as described earlier). This explanation is supported by the observation that the hypertension can be successfully treated with venodilating drugs, but not arterial dilating drugs such as nitroglycerin.[43,45]

The situation in patients with autonomic nervous system failure complements substantial other evidence indicating that, in addition to gravitational forces, activation of the sympathetic nervous system (SNS) is a major mechanism for redistribution of blood volume, especially by affecting splanchnic vascular capacitance.[18,20-21,29]

5. Sympathetic nervous system activity to the splanchnic vascular bed in hypertension

Sympathetic nervous system activity is elevated in some humans with hypertension,[29,46-48] and in several experimental hypertensive animal models[49-51] (for example, DOCA-salt and spontaneously hypertensive rats). The elegant work of Esler and colleagues[48] using norepinephrine spillover techniques revealed that sympathetic activity was increased in specific vascular beds in human essential hypertension: the majority of the increase was found in the heart and kidneys. Moreover, this increase was most prominent in borderline or pre-hypertensive subjects, which further suggests that over activity of the SNS is a key component in the *development* of hypertension.[47]

Splanchnic veins and venules located in the abdominal region, which directly supply blood to the central venous compartment, are richly innervated by the SNS.[21,52-53] Activation of the SNS increases the vascular tone of smooth muscle surrounding these highly compliant, large blood-storing veins.[54] As discussed above, this allows for mobilization of blood toward the central venous compartment and heart. Consequently, sympathetic venoconstriction of the splanchnic veins can be an important regulator of cardiac filling and arterial pressure.[18,20-21,53-54]

There is evidence of activation of the splanchnic SNS in hypertension.[18,20-21,53-54] A significant increase in splanchnic nerve activity, measured by direct nerve recordings in conscious rats, was found in angiotensin II-induced hypertension.[55] A

sympathetically mediated increase in venous motor tone was also found during the development of angiotensin II-salt hypertension in rats.[51,56] Indirect evidence found in human borderline hypertension showed vascular resistance to be elevated in the hepatosplanchnic circulation, (a region where sympathetic activity is often elevated), before occurrence in other vascular beds.[57] These findings suggest the importance of sympathetically mediated constriction of splanchnic capacitance vessels during the developmental stages of hypertension.

Splanchnic arteries and veins are innervated by sympathetic nerves through the paravertebral and prevertebral ganglia.[58] Postganglionic neurons innervating the splanchnic vascular bed are located within the celiac and superior mesenteric ganglia, which are fused and referenced as the celiac plexus.[59] Therefore, sympathetic input to the splanchnic vascular via the celiac plexus has the potential to decrease splanchnic venous capacitance. In a paper from 1941, successful treatment of human hypertension was achieved by surgically removing the celiac ganglia (celiac ganglionectomy, or CGX).[60] In the DOCA-salt and ANG II experimental animal models, CGX has shown to reduce arterial pressure.[56] Studies in humans with essential hypertension demonstrated that surgical section of the splanchnic nerves also is effective in treating essential hypertension.[61] Additionally, celiac plexus neurolysis for the treatment of pancreatic malignancies in humans often results in transient but severe hypotension.[62] Finally, chronic electrical stimulation of the splanchnic nerves in dogs produced sustained hypertension.[63-64] Together these findings indicate that splanchnic

sympathoactivation may be an important factor in the development of hypertension. In my experiments, CGX will be used to examine the contribution of splanchnic sympathetic nerves to blood volume redistribution and hypertension development in rats.

6. The use of bioimpedance to estimate blood volume distribution

Changes in total circulating blood volume can be a key factor in diseases such as congestive heart failure[65], in medical conditions such as syncope[66], and possibly in hypertension (as discussed earlier). Accurate measurement of total circulating blood volume nevertheless is difficult and remains a clinical challenge.[67-68] But, most importantly, methods used to measure total circulating blood volume do not take into the account the *distribution* of blood within the circulation. The central hypothesis of my research is that *both* the total amount of blood within the circulation, and its relative *distribution* in high and low compliance vascular compartments (veins and arteries, respectively), can contribute to hypertension development.

A significant obstacle to testing this hypothesis is that there is currently no method available that allows direct quantification of arterial blood volume or venous blood volume. Arterial and venous volume *fractions* have been estimated in individual organs using complicated plethysmographic or imaging methods[69-70], however these approaches are not applicable for use in the whole animal. Instead, investigators

have taken advantage of the fact that venous blood volume is much larger than arterial volume in all tissues, and that some vascular regions (splanchnic organs) have much larger blood storing capacities than others (muscle, skin, heart).[14,20-21,49,56,58] Thus, with a few exceptions (e.g. heart) a measured decrease in organ size, weight, or total blood content is generally accepted to reflect primarily a decrease in venous blood volume. And as discussed earlier, the high capacitance splanchnic organs are understood to represent the bulk of the "peripheral" venous compartment. Therefore, measurements of regional blood volume (especially in the splanchnic organs) are used as a surrogate for direct measurement of venous volume.

Blood volume distribution within multiple compartments of the circulation can be determined in animals and humans using a variety of techniques[67-68,71], but the best approach currently available is blood pool scintigraphy.[72] This method allows for direct tracking of radiolabeled blood cells in various vascular regions. However, this approach does not offer repeated or continuous measurement of changes in blood volume over extended periods of time in the same animal. That is why for these studies I chose to use bioimpedance, a well-established method to estimate changes in vascular volume within specific compartments of the circulation.[73-74] Specifically, this method offers the possibility to make continuous (or repeated) measurements in conscious rats.

Bioimpedance methodology is based on a simple model that regards the body (human or animal) as a cylindrical conductor, composed of various electrical compartments comprising simple resistors and capacitors.[75] Bioimpedance is measured by the conductive response of the body to an externally applied low voltage electrical current and the resulting resistance or impedance to this current.[73-75] Bioimpedance is inversely proportional to the estimated amount of total body water present.[74,76-77] As impedance increases, the volume of estimated total body water decreases and vice versa.

Segmental bioimpedance can be used to monitor fluid volume changes within specific anatomic regions.[76] Moreover, the measurement of bioimpedance is routinely used as a surrogate for shifts in *blood volume.*[78-80] Total body water is composed of both extracellular and intracellular fluid. Intracellular fluid is rarely mobile within the circulation. Thus it is the extracellular fluid, composed mainly of blood and interstitial fluid that can be readily mobilized within a body region. Blood volume in most regions of the body is much larger than the interstitial fluid volume. Therefore, changes in impedance can be used as a proxy for changes in blood volume.[74-75] Thus, our hypothesis was addressed using the technique of bioimpedance. For this research, the anatomical regions used for segmental bioimpedance were: (1) the chest, representing the central venous compartment, heart and lungs; and (2) the abdomen, representing the splanchnic region, which is a component of the peripheral venous compartment.

Although bioimpedance has been extensively validated in humans, the application of bioelectrical impedance in experimental animal models has been sparse, especially in conscious unrestrained animals. In order to utilize this technique, the technique would need to be used in conscious and free-moving laboratory rats to make repeated, within-animal estimates of blood volume redistribution between the peripheral (splanchnic) and central venous compartments.

7. Animal Model: Deoxycorticosterone acetate-salt Hypertension

Over the past 50 years, numerous experimental models of hypertension have been developed, predominantly in rats.[50] Because the etiology of essential hypertension is heterogeneous, it is nearly impossible for any one experimental rat model to encompass all facets of this disease. For this study, I chose to use the deoxycorticosterone acetate (DOCA)-salt hypertensive rat model, which has been shown to be an effective model to study volume-dependent hypertension. This hypertensive model involves the chronic treatment of rats with the mineralocorticoid, DOCA, in combination with a high salt (1% NaCl) intake via the drinking solution, and unilateral nephrectomy. The model is considered a prototypical example of salt-sensitive hypertension.[81] The concept of salt-sensitivity refers to the finding that some individuals exhibit an exaggerated increase in blood pressure when consuming high dietary salt, whereas others show little change in blood pressure.[82] The former individuals are referred to as 'salt-sensitive'. Thus, the concept of salt sensitivity provides an explanation for the

generally reproducible connection between high salt intake and elevated blood pressure in large populations of people in which many individuals have normal blood pressure despite daily consumption of large amount of salt.

DOCA is a potent mineralocorticoid that is known to cause significant sodium and water retention[83] by reducing the ability of the kidneys to excrete sodium.[84] The effectiveness of DOCA in elevating blood pressure is directly proportional to the salt intake of the animal.[84] Sodium is the major cation in the extracellular fluid and its excretion is regulated almost exclusively by the kidney.[27] The level of total body sodium, which is increased in the DOCA-salt model, contributes significantly to the osmolality and volume of the extracellular fluid. Thus, the regulation of total body sodium has become synonymous for volume regulation.[27] Therefore, DOCA-salt has commonly been classified as a volume-dependent model of hypertension.[85]

It is widely accepted that DOCA-salt treatment causes an increase in total body water with proportional increases in circulating fluid volume.[84] Furthermore, recent work in dogs with renal hypertension[86] shows that total body water is a major determinant of arterial pressure. Thus, it is axiomatic that DOCA-salt treatment produces an elevation in arterial blood pressure by increasing *total* fluid volume within the circulation.

Nevertheless, recent data has revealed that total body water is not always directly associated with arterial pressure. For example, Titze et al[81,87-88] have shown that

sodium retention can take place free of water accumulation, including in DOCA-salt hypertension in rats. High sodium intake in DOCA-salt treated rats resulted in an increase in total body sodium content by 50% within 5 weeks; however only ~ 20% of the sodium accumulated led to volume retention.[87] As a result, only moderate increases in total body water was achieved despite massive sodium retention. The excess sodium was shown to be stored in osmotically inactive and/or osmotically neutral sodium storage sites, which did not play a part in volume expansion, yet the animals were hypertensive.[87-88] These results argue against the traditional volume-dependent view held of the DOCA-salt model of hypertension, and provide clear evidence that an overall increase in total body sodium does not always increase arterial pressure through blood volume expansion.

It has also been shown that DOCA-salt increases venomotor tone by activation of the SNS.[49,54,85] As described earlier, the SNS is an active regulator of splanchnic venous capacitance and thereby contributes to the redistribution of blood volume from the peripheral to the central venous compartment. A study comparing DOCA-salt rats to sham-operated rats revealed no difference in total circulating fluid volume between the two groups yet the DOCA-salt rats were hypertensive.[85] It can be speculated from this study that DOCA-salt treated rats had an increase in central blood volume due to a decrease in peripheral venous capacitance, although this was not measured directly. In a study in sheep, however, mineralocorticoid-salt treatment caused an immediate and sustained increase in central venous pressure

and stroke volume without any change in total body fluids, strongly suggestive of peripheral to central blood volume redistribution. [89]

In a well-devised study, *the experimental question should dictate the experimental model.*[50] The DOCA-salt experimental rat model offers this opportunity to address the experimental question of vascular volume, and its regional redistribution affect on arterial pressure regulation.

8. Summary and Overall Importance of Current Research

The overall hypothesis of this work is that volume redistribution within the circulatory system (from peripheral towards central venous compartment and into arteries) affects arterial pressure during the onset of hypertension. In order to address this aim, the DOCA-salt experimental rat model and the technique of segmental bioimpedance was used. The DOCA-salt model has long been cited as a volume-dependent model of hypertension. (i.e. increased blood pressure is caused by increased body fluid volume.) Overall volume expansion within the circulatory system, presumably caused by alterations in the pressure-natriuresis mechanism, is well documented as contributing to the regulation of arterial pressure.[24,27,29] However, studies have also shown that the initial elevation of arterial pressure in early stage essential hypertension may occur by another mechanism—a redistribution of fluid volume—which is independent of overall circulatory volume expansion.[16,20-21,49] This translocation of blood volume from the peripheral to

central venous compartment is likely brought about by a decrease in peripheral venous capacitance due to activation of the SNS, specifically within the splanchnic veins. [13-14, 18,20-21,49]

Segmental bioimpedance was used to estimate changes in blood volume between the peripheral and central venous compartments in conscious unrestrained rats treated with DOCA and salt. Unlike other methods to determine blood volume distribution, the bioimpedance method allows for repeated (or continuous) measurement of regional blood volume within the same animal over an extended time. Tracking fluid movement during the transition from normal blood pressure to hypertension and back to normal pressure could provide significant insight into the role of blood redistribution (and the mechanism driving this movement, presumably increased sympathetic tone to the splanchnic veins) in the development of hypertension. Furthermore, I purpose that this was due to an increase in sympathetic tone to the splanchnic veins.

My research is important as it provides additional insight into one possible mechanism, blood volume redistribution, which has been cited in contributing to the development of hypertension, but no direct evidence has been shown to support it. It is well established that hypertension is the leading cause of cardiovascular death and disability worldwide. This is forecasted to continue[1-2] (by 2025, 1.5 billion people are predicted to be hypertensive.)[2] Thus, there is much to be gained by a further understanding of the mechanisms that occur during the early development

of hypertension. This is crucial in delaying or preventing the stable form of this continuous disease, as well as, reducing end organ damage and the significant economic burden that accompanies this lifetime condition.

Central Hypothesis

A factor in the development of DOCA-salt hypertension is reduced splanchnic vascular capacitance leading to blood volume redistribution from the high-compliance splanchnic vasculature (abdomen) toward the central circulation (heart and lungs), and ultimately into the less compliant arteries.

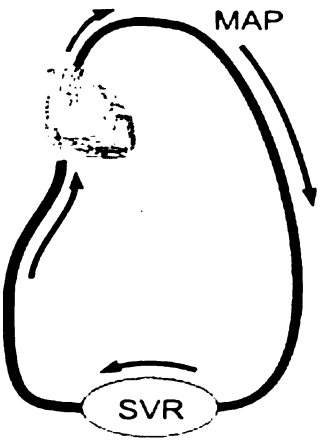

Figure 1: The circulatory system is a semi-closed loop. The volume and pressure contained within the arterial system, as well as, the systemic vascular resistance (SVR) are all components in determining the mean arterial pressure (MAP).

Specific Aims

I. **Specific Aim 1:** To establish a bioimpedance method in a conscious reely moving laboratory rodent to estimate circulatory fluid volume shifts.

II. **Specific Aim 2:** Use chronic bioimpedance measurements to determine the changes in blood volume during the onset of DOCA-salt hypertension.

III. **Specific Aim 3:** Determine if activation of the sympathetic nervous system contributes to redistribution of blood volume during the development of DOCA-salt hypertension.

General Methods

1. Animals

Male Sprague-Dawley rats (Charles River Laboratories, Portage MI) weighing 300-325 grams and normotensive at the beginning of the study were used in all experiments. The Michigan State University Committee on Animal Use and Care approved all protocols. The rats were housed in groups of 3 in Plexiglas® cages in a temperature and humidity controlled room with a 12-hour light/dark cycle and allowed to acclimatize for a week prior to any surgical procedures. Rats were given standard pelleted rat chow (8640 rodent diet; Harlan/Teklad, WI) and water ad libitum.

2. General Anesthesia

All surgical procedures were performed using an inhalation anesthetic agent (isoflurane) delivered through a nose cone. The induction chamber contained a 4% isoflurane in oxygen mixture. The animals were maintained during the surgery by 2% isoflurane in oxygen mixture. Rats were recovered from anesthesia under close observation on a heating pad.

3. Analgesia and Antimicrobial Prophylaxis

Post-operative analgesia and antimicrobial prophylaxis were established by administration of enroflaxcin (5 mg/kg IP), ticarcillin-clavulanate (200 mg/kg IP) and carprofen (5 mg/kg SQ) respectively. Post-surgery analgesia was continued for an additional 5 days with carprofen (5mg/kg SQ).

4. DOCA-salt Hypertension Protocol

All rats underwent a unilateral nephrectomy of the left kidney to reduce renal mass before the start of the experiment. Under general anesthesia, a left lateral abdominal incision was used to access the left kidney. The left kidney was exteriorized, left renal vessels and ureter were tied off, and left kidney was removed. The incision was sutured closed in layers. Following 7 days of surgical recovery and a 5-day control period, all rats were randomly divided into their respective groups. Rats that were to receive a DOCA pellet were allowed free access to saline (distilled water containing 1% NaCl and 0.2% KCl) for 3 days. Sham-operated rats (SHAM) were allowed free access to distilled water throughout the study. After 3 days, a DOCA pellet (150 mg/kg) impregnated with silicone rubber was implanted subcutaneously on the dorsal side of the DOCA-treated group of rats while the SHAM group underwent a similar subcutaneous surgery but did not receive an implant. After 5 days, the DOCA-treated group were taken off saline drinking solution and given free access to distilled water for the remainder of the experiment.

5. Bioimpedance Electrode Implantation

The bioimpedance technique was achieved by permanently implanting 6 stainless steel electrodes (Plastics One, VA) subcutaneously. While rats were under general anesthesia, electrodes were implanted subcutaneously through a 1 cm incision made in the skin above the specific anatomical location as described below. The electrodes were tied into the underlying muscle with suture and the incision was sutured closed. All the electrodes were exteriorized between the scapulae and

were connected to a swivel arm that allowed the rat free-movement throughout the cage. All electrodes were in place before the start of the experiment. The rats were given 7 days to recover from electrode implantation.

6. Bioimpedance Measurements

Bioimpedance was used to detect internal volume shifts chronically in conscious, free moving rats. The electrodes were connected to a two-channel tetrapolar high frequency impedance meter (Thrim®) that introduced a high frequency, low amperage, constant current, which was insensible to the rat. Current was injected into 2 electrodes positioned dorsally at the base of the head and tail. These electrodes represent the driving electrodes through which current (excitation:1mA and frequency:51.2kHZ)) is injected into the rat. In addition, 4 electrodes implanted at precise pair-wise distances across the thorax (midline xiphoid process), and abdomen (iliac crest) represents the detecting electrodes, which measure the voltage drop across these anatomical segments of interest: thorax and abdomen.

Impedance or resistance to this high frequency current is inversely proportional to the amount of fluid volume in the tissues between the recording electrodes. As compartmental fluid volume decreases, measured impedance increases. Each rat was measured for abdominal and chest impedance daily for a duration of 20 minutes at the same time every morning while housed undisturbed in their home cage. An average for the recorded 20 minutes, for both abdominal and chest impedance, were derived by a commercially available data acquisition program (Powerlab, ADinstruments) per rat. Each daily impedance average was converted to

estimated compartmental fluid volume value by the following mathematical conversion: 10 + 1/bioimpedance recording, which was used to avoid very small values. The inverse of bioimpedance is sometimes referred to in the literature as "admittance". Bioimpedance and impedance are used interchangeable throughout this thesis.

7. Radiotelemetry Transmitter Implantation:

Radiotelemeter transmitters for measuring arterial pressure were implanted in all rats before the start of the experiment. Under general anesthesia, the tip of the transmitter catheter was inserted into the abdominal aorta by way of the femoral artery. The body of the transmitter was placed in a subcutaneous pocket along the abdomen but below the abdominal impedance electrodes, so as not to interfere with bioimpedance measurements. The incision was closed in layers. All animals were given 7 days to recover.

8. Arterial Pressure Measurements

Rats were housed in individual Plexiglas® cages and placed on radiotelemetry receivers (RPC-1, DSI). The arterial blood pressure was recorded for 10 seconds every 10 minutes throughout the experiment. A commercially available radiotelemetry data acquisition program (Dataquest, DSI) was used to remotely monitor the arterial pressure. The radiotelemetry system did not interfere with bioimpedance recordings during the study.

9. Celiac Ganglionectomy

Under general anesthesia, a ventral midline abdominal incision was performed and the small intestines were gently retracted and placed on warm ticarcillin-clavulanate (200mg/kg) and enroflaxcin (5mg/kg) soaked gauze. The celiac plexus located between the aorta, celiac artery and mesenteric artery was dissected free and removed. The small intestines were placed back into the abdominal cavity and lavaged with warm saline. The midline abdominal incision was sutured closed in layers. The SHAM group underwent a sham operation that was performed by accessing and exposing the celiac plexus only.

10. Confirmation of Regional Denervation

At the completion of the protocol the rats were sacrificed by an intraperitoneal injection of sodium pentobarbital (100mg/kg). The liver, spleen, small intestines and right kidney, which represent organs that are innervated by the celiac ganglia in the splanchnic region, were harvested from each rat. The tissues were weighed and immediately frozen in liquid nitrogen. All tissues were stored at -80^{o}C for later analysis. High performance liquid chromatography (HPLC) analysis was used to measure norepinephrine (NE) content in each tissue, as an index of the density of sympathetic innervation of the tissues. Data was reported as nanogram of NE per gram of tissue. The Michigan State University Department of Pharmacology and Toxicology HPLC core facility performed these assays.

11. Plasma Volume Measurements

All rats were chronically instrumented with silicone-tipped catheters into the femoral artery and vein for Evans blue administration and blood sampling. The two catheters were exteriorized between the scapulae of the rat into a stainless steel spring that was attached to the rat at one end by a loosely fitted rubber jacket (Instech Solomon), and the other end to a swivel arm at the top of the cage. This allowed for free movement and access to the catheters without handling or disturbing the rat. The rats were housed in individual Plexiglas® cages and recovered for 5 days. All catheters were flushed daily with a heparin-saline solution. Plasma volume was estimated with the use of 10-minute distribution volume of Evans blue dye. Arterial blood (0.6ml) was collected at baseline (before Evans blue dye injection) and 10 minute after intravenous Evans blue dye (1mg/ml in saline) injection. The Evans blue dye will remain in the vascular space during this timeframe. All blood samples were collected in heparinized EDTA tubes and centrifuged. The plasma was collected. Evans blue dye concentration was determined by spectrophotometry and absorbance was read at 650nm. The dye concentrations in the collected samples were measured by using a standard curve of Evans blue dye solution in the plasma of the baseline sample.

12 Animal Euthanasia

At the conclusion of each study, an intraperitoneal injection of sodium pentobarbital (100 mg/kg, i.p.) was administered. This adheres to the Michigan State University Animal Care and Use Committee guidelines for euthanasia.

13 Statistical Methods

Mean arterial pressure and impedance data were statistically analyzed by one-way repeated measures ANOVA. When applicable, post-hoc multiple comparisons using Dunnett's procedure (GraphPad, Instat) was used to compare all days to control period day 2. A p-value <0.05 was considered significant. All results are reported as mean $\pm$ SE.

Specific Experimental Protocols and Results

I. **Specific Aim 1: To establish a bioimpedance method in a conscious unrestrained laboratory rat to estimate circulatory fluid volume shifts during the onset of hypertension.**

Background:

Bioimpedance is a novel technique used to determine estimated fluid volume changes within specific regional vascular compartments.[73-74,76] In this study, I used bioimpedance to measure changes in fluid volume within the abdominal region, representing the highly compliant splanchnic vascular compartment, and the thoracic region, representing the lesser compliant central vascular compartment. These measured changes in fluid volume were used as a proxy for blood volume shifts into and out of these vascular regions. [74-80] Although other methods of blood volume distribution are available, bioimpedance was used because it allows for repeated measures in individual animals.

It is well documented that gravity, as illustrated by cardiovascular responses produced by postural changes (i.e. tilting), is a potent regulator of blood volume distribution within the vascular system.[13,15,90-92] During passive postural tilts, the head is moved above (head up tilt; HUT) or below (head down tilt; HDT) the level of the heart. During initial changes in posture, blood volume is shifted between the peripheral and central (heart and lung) vascular compartments by gravitational forces; later, compensatory mechanisms (e.g. baroreceptor reflex and neurohumoral factors) also contribute to maintaining homeostasis of the cardiovascular

system.[92,93] Therefore, postural tilting is one reliable way to induce blood volume shifts between specific vascular compartments. I used this approach in order to validate the bioimpedance technique in rats.

During passive head-up tilt (HUT) gravity pulls blood out of the chest towards the compliant veins of the abdomen and lower extremities. As a result, central blood volume is reduced. This leads to a decrease in venous return and cardiac output, resulting in a transient lowering of mean arterial pressure.[13,92-93] The unloading of arterial baroreceptors causes sympathetic vasoconstriction of veins and arteries in the peripheral compartment. The result is reduced peripheral compartment capacitance and redistribution of blood back towards the central venous compartment, heart and arteries to maintain arterial volume and pressure.

During HUT in rats I anticipated an initial increase in impedance in the chest (decrease in fluid volume) as blood moves out due to gravitational change. Impedance in the abdomen should decrease (increase in fluid volume), as blood gathers in the veins of the large capacity splanchnic region and lower limbs. These impedance changes should eventually stabilize due to activation of compensatory mechanisms to maintain cardiovascular homeostasis.

During head-down tilt (HDT), gravity works to move blood from the abdomen and other peripheral vasculature into the central venous compartment. This increases central venous compartment volume, venous return and cardiac output resulting in a transient increase in arterial pressure.[16,92-93] The cardiovascular control system

compensates by reducing sympathetic vasoconstriction (reduced renin-angiotensin system activity and other mechanisms that help to maintain homeostasis). During HDT in rats, I anticipated an initial decrease in impedance in the chest (increase in fluid volume) as blood gathers in the central compartment. Impedance in the abdomen should increase (decrease in fluid volume) as blood shifts towards the central venous compartment due to gravity. Although these are expected findings, a number of studies done in humans reported that stroke volume, cardiac output and thoracic admittance show minimal or no change in response to HDT.[78,92-93] Presumably this is because the central venous compartment 1) has relatively low compliance, 2) is maintained near maximal volume even in upright humans, and 3) is rapidly emptied of excess volume by cardiac pumping.

Hypothesis:

The bioimpedance method will detect appropriate directional changes in central and peripheral venous compartment blood volume during HUT and HDT in rats.

Protocol:

Electrodes for segmental bioimpedance measurements were implanted in a rat as described under General Methods. While under general anesthesia, the instrumented animal was strapped supine to a tilt table board. It was maintained in a supine position during a 10-minute control period, to establish steady state bioimpedance values. This was followed by ~90° HUT for 5 minutes. The animal was returned to the supine position to re-establish initial steady values, which

followed a ~90° HDT for 5 minutes. Only bioimpedance was recorded during this protocol.

Results:

The results for HUT are presented in figure 3. During HUT, estimated fluid volume decreased in the thoracic compartment while it simultaneously increased in the abdominal compartment. When the rat was returned to the supine position, estimate fluid volumes returned to their control values within about 2 minutes. The outcome for HDT is shown in figure 4. During HDT, estimated fluid volume increased in the thoracic compartment while it simultaneously decreased in the abdominal compartment. When the rat was returned to the supine position, estimate fluid volumes had not yet returned to their control values within 2-3 minutes. It is not possible to determine from these measurements the absolute volumes of fluid moving in and out of the compartments during tilting. However, to provide additional information on the sensitivity of the bioimpedance technique, Figure 5 shows higher gain recordings of abdominal and thoracic impedance in the animal in the supine position. Note that a steady, rhythmic impedance recording was obtained that which is consistent with the phases of the animal's inspiration and expiration.

Conclusion:

The bioimpedance measurements for HUT and HDT in this study was consistent with the volume shifts expected during postural tilt, as previously described. From the literature, stroke volume is shown to differ by ~8% during inspiration and

expiration[94] and the normal central blood volume in a 330g rat is ~4.0 ml.[95] Using this difference as an index of alterations in central blood volume during respiration—and the data in Figure 5—allows me to conclude that the bioimpedance method is able to detect within-animal changes in compartmental volume of less than 0.3 ml. Since the volume of blood that can be transferred in and out of the splanchnic and abdominal compartments is on the order of 2-3 ml in the rat (i.e. 10-15% of total blood volume), the bioimpedance method has adequate sensitivity for the proposed studies. From these findings, I conclude that I have established a bioimpedance technique that can be used in a conscious animal for repeated (or continuous) measurements of physiologically relevant changes in compartmental fluid volume.

II. **Specific Aim 2: To use bioimpedance to estimate blood volume changes in the abdomen (peripheral venous compartment) and the chest (central venous compartment) during the onset of DOCA-salt hypertension in conscious rats.**

Background:

The natural progression of essential hypertension has been shown to be associated with an increase in central blood volume.[12,16-17] This excess of blood in the central compartment could increase cardiac filling pressure; and by the Frank-Starling mechanism cause more blood to be ejected into the low compliance arterial system. This would result is modest arterial distension and an increase in arterial pressure. Two mechanisms, as described earlier, can increase blood in the central venous compartment resulting in an elevation in blood pressure during hypertension development.[14,20] One mechanism is by increasing total blood volume contained in the circulatory system. This could be achieved by an alteration in the pressure-natriuresis mechanism, thereby causing the kidneys to conserve salt and water, resulting in an increase in total and central blood volume.[24-25] This is the prototypical explanation for DOCA-salt hypertension, i.e. an increase in total body fluid producing increased arterial pressure.[86]

The other possible mechanism is by a decrease in peripheral venous capacitance, thereby mobilizing stored blood towards the central compartment (heart and lungs) and into the arteries. This transfer of additional blood from peripheral to central venous compartment would result in an elevation in arterial pressure without a change in total blood volume.[14,16,18,] Vasoconstriction of splanchnic arteries and

veins can greatly contribute to this redistribution of blood volume.[20,21] Recent studies using the DOCA-salt model, as previously described, suggest that the onset of hypertension may also occur via this mechanism.[16] However, to date there is no direct evidence for blood volume redistribution during the development of DOCA-salt hypertension.

Therefore, bioimpedance was used in the DOCA-salt rat model to further explore changes in blood volume within the peripheral and central venous compartment during hypertension development. Measurements were obtained daily in conscious rats. The chance to determine estimated compartmental blood volume during the transition from normal arterial pressure to hypertension and back to normal pressure is important to test my central hypothesis, i.e. a decrease in peripheral venous capacitance and a redistribution of blood volume, which is particularly important in the *development* of DOCA-salt hypertension.

Hypothesis:

During the onset of DOCA-salt hypertension, peripheral venous capacitance is reduced and blood volume is redistributed from the abdomen (peripheral venous compartment) to the chest (central venous compartment) and ultimately into the arterial circulation, thereby increasing arterial pressure.

Protocol:

Impedance electrodes were implanted in two groups of rats, DOCA-treated and SHAM. All rats underwent uninephrectomy and had a radiotelemeter inserted for blood pressure measurement, as described in the Methods section. Bioimpedance

was individually recorded in each rat daily for 20 minutes during the study as described in the methods. Blood pressure was recorded continuously throughout the study. The protocol for this study is outlined below in figure 2.

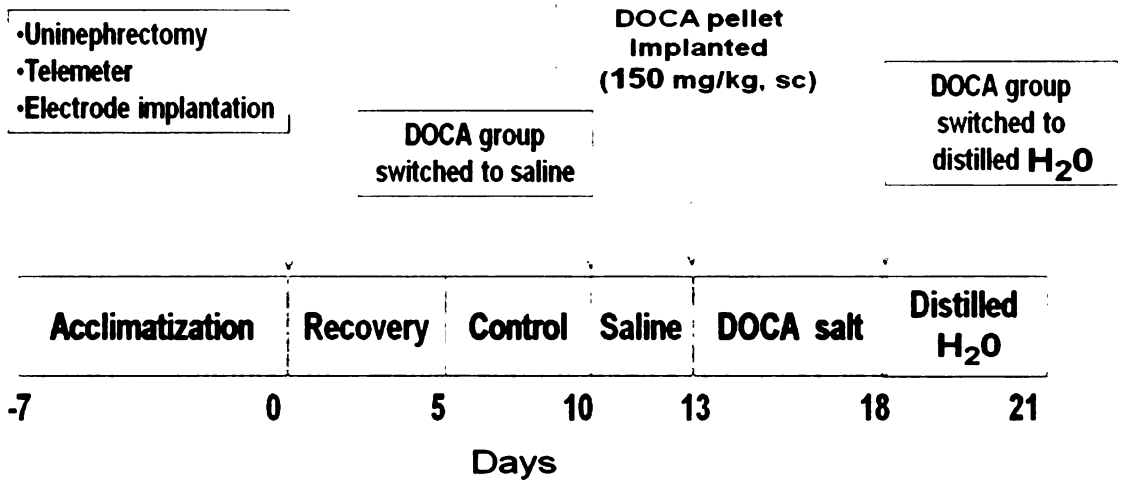

Figure 2: The protocol for the development of DOCA-salt hypertension in SHAM and DOCA-salt treated rats.

Results:

A total of 15 rats were studied in two groups (SHAM and DOCA). The mean arterial pressure response for both groups is shown in figure 6A. SHAM rats exhibited a stable blood pressure throughout the protocol. High salt intake due to saline drinking caused a small, but non-significant increase in MAP (~7 mmHg). The combination of DOCA and salt caused a rise in MAP that was statistically significant by days 4-5 of DOCA treatment. MAP reverted back to control values within 3 days after the rats were switched back to distilled water to drink.

Figure 6B represents the estimated fluid volume in the thorax for both groups. Neither the DOCA nor SHAM rats showed a detectable change in thoracic fluid volume throughout the course of this study.

The estimated fluid volume in the abdomen for SHAM and DOCA is presented in figure 6C. SHAM rats showed no change in fluid volume in the abdomen during the course of the study. High salt intake alone due to saline drinking caused no detectable change in abdominal fluid volume. However, a statistically significant decrease in abdominal fluid volume was detected as early as one day after beginning combined treatment with DOCA-salt; this reached statistical significance on days 4-5 of combined treatment. Abdominal fluid volume returned to near initial control period values when the DOCA-salt treated rats were switched to distilled water to drink.

Conclusions:

Blood volume redistribution occurred early during the development of DOCA-salt hypertension. This was shown by a decrease in estimated abdominal fluid volume, suggesting a decrease in peripheral venous capacitance. When DOCA-treated rats were switched back to distilled water, representing a recovery to a normal salt diet, both mean arterial pressure and abdominal estimated fluid volume reverted back to initial control values. These findings support the hypothesis that peripheral venous capacitance decreases during the onset of hypertension.

Interestingly, during the onset of hypertension there was no detectable change in estimated fluid volume in the chest (central venous compartment). This finding did not conform to the expected pattern of a shift in peripheral venous blood to the central venous compartment. Nevertheless, the results were not a total surprise, considering the previously described human data on head down tilt, where no change in thoracic fluid volume was seen.[92] One hypothesis for this finding is that the bioimpedance technique is not sensitive enough to detect small changes in fluid content within the chest region. However, in light of the data from Specific Aim 1, this possibility seems unlikely considering the high sensitivity of the bioimpedance technique to detect volume changes with regards to breathing as observed in figure 5.

Another alternative hypothesis is that the lack of measureable blood volume change in the chest is due to the heart. This sensitive "demand pump" rapidly transfers most of the blood volume redistributed from the abdominal region into the arteries and other parts of the circulation, possibly making impedance measurements undetectable.

In summation, the overall results are consistent with the idea of blood volume redistribution, out of the abdominal region into the central circulation, as a contributing cause in hypertension development, but do not prove a cause-and-effect relationship.

III. **Specific Aim 3: Determine if activation of the sympathetic nervous system contributes to the redistribution of blood volume during the development of DOCA-salt hypertension.**

Background:

Splanchnic veins and venules are innervated by the SNS.[52-53] Sympathetic venoconstriction of these vessels reduces venous blood volume and overall vascular capacitance.[14,20-21] This could result in a redistribution of stored blood into the central circulation and ultimately increase arterial pressure. In addition, in DOCA-salt hypertension there is an increase in splanchnic venomotor tone mediated by increased sympathetic activity.[21,54] All the above indicates that increased SNA to the splanchnic circulation could be one mechanism involoved in DOCA-salt mediated hypertension. The majority of sympathetic innervation to the splanchnic vasculature in the rat is through the celiac plexus.[52] Surgical removal of this plexus (celiac ganglionectomy, CGX) was used to investigate the contribution of sympathetic nerve activation to the redistribution of blood volume during the development of DOCA-salt hypertension in the rat.

Hypothesis:

Selectively removing sympathetic nerve activity to the splanchnic circulation by CGX will attenuate blood volume redistribution and the development of DOCA-salt hypertension.

Protocol:

Surgical CGX or SHAM-operation (SGX) were performed during the same surgery as the impedance electrodes, radiotelemeter and uninephrectomy. Animals were given 7 days to recover before the start of the experiment. The protocol and measurements made in this study were otherwise the same as rats in the DOCA group in Specific Aim 2 shown in figure 2. At the conclusion of the study, the splanchnic organs represented by the liver, spleen, small intestines and right kidney were harvested and measured for norepinephrine content by HPLC to confirm effective splanchnic denervation.

Results:

A total of 14 rats were studied in two groups. Tissue norepinephrine content of the splanchnic organs, verifying successful celiac ganglionectomy, is shown in figure 7. Celiac ganglionectomy nearly abolished tissue norepinephrine content in the spleen, small intestine and liver. The effect of CGX on MAP responses to DOCA-salt is presented in figure 8A. First, rats with CGX had reduced MAP compared to sham rats throughout the protocol. Second, both CGX and SGX rats exhibited modest and similar increases in MAP (~5 mmHg) when on high salt intake due to drinking saline. However, when both groups were given DOCA and salt, MAP increase was statiscially significant in SGX on day 2 through 5, but only minimally in CGX, rats. Switching back to distilled water to drink reduced MAP back towards control period values in SGX rats, and minimally reduced MAP in CGX rats. Data on estimated thoracic fluid volume are shown in figure 8B. There was no observed change in thoracic fluid volume for either group throughout the protocol. Data on estimated

abdominal fluid volume are presented in figure 8C. There was no change observed in estimated abdominal fluid volume for either group throughout the study. This is consistent with my hypothesis for CGX rats but a surprise for the SGX rats.

Because of the very *unexpected lack of change* in the abdominal fluid volume data in rats that underwent abdominal surgery (CGX and SGX), I compared their data to those from rats that did not have abdominal surgery (Figure 9). Based on our bioimpedance measurements, abdominal fluid volume in rats that underwent abdominal surgery for celiac ganglionectomy or sham operation appeared to be *lower* than in rats that did not have abdominal surgery. This was an unexpected finding. Thoracic fluid volume in rats that underwent abdominal surgery was *higher* than in rats that did not have abdominal surgery. This was also an unexpected finding.

Conclusion:

From this study, I conclude that CGX selectively disrupts sympathetic innervation to the splanchnic organs and impairs development of DOCA-salt hypertension. In studies described under Specific Aim 2, a decrease in estimated abdominal blood volume occurred with the development of DOCA-salt hypertension. In this study, DOCA-salt treated rats with CGX had no detectable change in estimated abdominal fluid volume. This was expected according to my hypothesis, because removing sympathetic innervation by CGX should prevent constriction of the splanchnic veins and the resulting redistribution of blood volume towards the heart and arteries. However, the sham surgery rats that were treated with DOCA-salt also showed no

change in abdominal fluid volume. This was a surprise. What was expected was a decrease in abdominal fluid volume corresponding with the observed increase in arterial pressure, as I reported in studies under Specific Aim 2. From these findings it appears that abdominal surgery, at least in the SXG rats, is in some way, altering blood volume regulation. The surgery, (abdominal incision along with blunt dissection of the celiac plexus) to disrupt splanchnic inveration is relatively minor, however, the process of locating the celiac plexus is fairly invasive, as all abdominal organs (stomach, intestines, spleen) need to be carefully retracted and excised. It is possible that tissue damage can occur even with the up most caution. Thus, the addition of pro-inflammatory and anti-inflammatory mediators and the governing inflammation process due to abdominal surgery and manipulation of the gastrointestinal organs could possibly explain the unanticipated results in the sham surgical animals. In a previous study, simple laparotomy (abdominal incision) and gentle handling of the stomach, intestines and spleen resulted in microvascular changes within these tissues.[96-98] These changes included an increase in vascular permeability, plasma extravasation, as blood moved from the capillaries to the surrounding injured tissues. [96,98] The abdominal organs being handled during surgery are representative of the splanchnic organs (small intestine, spleen, and liver) and are reported to be most sensitive to surgical stress.[98] In fact, surgical stress has shown to increase vascular resistance within the vasculature of these splanchnic organs, but not uniformily[98], which could possibly explain the initial differences seen in the control period blood volume observed in the abdomen and thorax of surgery rats compared to non surgery rats in figure 9. A decrease in

arterial to venous blood transfer due to an increase in vascular resistance could result in a redistribution of splanchnic venous blood towards the heart to maintain the cardiac volume and filling pressure, but not enough to cause hypertension. From these findings and my results, I conclude abdominal surgery and the handling of the gastrointestinal organs alters the regulation of splanchnic vascular capacitance and blood volume.

To further investigate the surgical phenomena, plasma volume was measured in one DOCA-salt treated, SHAM-CGX rat, i.e. a rat subjected to abdominal surgery. The results are shown in figure 10. The development of hypertension in this rat (that underwent only abdominal surgery) was associated with an increase in total blood volume. Rats without abdominal surgery do not generally have an increase in plasma volume during DOCA-salt hypertension development, which is supported by the abdominal redistribution results in Specific Aim 2. This preliminary finding contributes to the idea that abdominal surgery significantly affects blood volume regulation. More experiments in DOCA-salt treated rats are needed to confirm and explain this abdominal surgery phenomenon.

In addition, plasma volume was measured in both surgery and non- surgery animals during the control period before the initiation of the DOCA-salt protocol. This data is shown in figure 11. In these preliminary results, abdominal surgery animals had a 10% lower plasma volume during the control period than non-abdominal surgery animals. This confirms with previous studies that plasma volume is decreased in

rats that undergo laparotomy and gastrointestinal organ manipulation[96-97,99] The loss of extracellular fluid is largely due to an internal redistribution i.e. plasma extravasation[98] (movement of plasma from the capillaries towards the surgical site) rather than loss from the surgical site , which is minimal. These findings further support my conclusion that abdominal surgery alone affects the control of blood volume.

When considering all of these preliminary plasma volume results, the data suggest that in animals that undergo abdominal surgery an increased total circulating blood volume contributes to the development of DOCA-salt hypertension. In DOCA-treated animals that do not undergo abdominal surgery, the early development of DOCA-salt hypertension appears to be due by a redistribution of blood volume. Overall, it is clear that decreased splanchnic blood volume *per se* is not *necessary* for development of DOCA-salt hypertension.

Overall Discussion and Conclusions

The onset of hypertension has often been characterized by an increase in central blood volume. This can be achieved by either an increase in total blood volume or by a redistribution of blood volume into the central compartment (heart and lungs). The purpose of this study was to investigate, in the DOCA-salt model, if a redistribution of blood volume caused by a decrease in peripheral venous capacitance contributes to this finding. The results of this study did confirm a

statistically significant reduction in the abdominal blood volume during the development of arterial pressure elevation. This supports my hypothesis that a redistribution of blood volume is important in the development of hypertension. However, when exploring the possible mechanisms contributing to this redistribution of peripheral venous volume, presumably by sympathetically mediated vasoconstriction of splanchnic veins, there were unexpected findings. For example, animals that had undergone CGX, a procedure that requires an incision in the abdomen (laparotomy) and handling of the abdominal organs to locate and denervate the splanchnic vasculature, showed expected results of attenuating increases in arterial pressure and no change in abdominal blood volume. At the same time, the control group (SGX/DOCA), which also underwent a similar abdominal surgery but no CGX, showed very unexpected results of no change in abdominal blood volume despite becoming hypertensive. It appears that abdominal surgery, at least for SGX, causes an alteration in blood volume regulation.

During surgery and manipulation of the gastrointestinal organs (stomach, intestines, spleen and often liver), it is possible that unforeseen tissue damage could result in local inflammation, leading to the accumulation of both cellular (neutrophils and leukocytes) and chemical mediators (such as histamine, pro-inflammatory cytokines and inducible nitric oxide) at the surgical site, resulting in a change in the microcirculation of these surgically manipulated tissues, as seen in previous abdominal surgery rats.[96,98]

One possible explanation for the alteration in the microvascular after surgery has been linked to nitric oxide (NO), which can be formed continuously in the vascular endothelium and neuronal tissue by the constitutive NO synthase (endothelial NO synthase and neuronal NO synthase, respectively).[100] Endogenous NO is known to play a key part in maintaining microvascular integrity during normal physiological circumstances.[101] It is important to note that NO can also be formed in blood vessels due to various pathological conditions, such as prolonged hemorrhage and septic shock, by the inducible form of nitric oxide synthase.[102-103] The physiologically generated NO by endothelial NO synthase beneficial microcirculatory effects are reported to attenuate vascular permeability, plasma extravasation and inflammation.[104] Therefore, rats that underwent laparotomy and gastrointestinal handling had a reduction in endogenous NO production and increase in vascular permability.[96,98] When these animals were given NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), there was an increase in plasma extravasation , which was made better with NO donor,-nitroso-glutathione.[96] This confirms the importance of endogenous NO in maintaining microvascular integrity during surgery. But, most importantly, verifies abdominal surgery and gastrointestinal organ manipulation disrupts the vascular endothelium by decreasing NO. While this is only one mechanism out of many that could occur during abdominal surgery and gastrointestinal organ handling, further investigation into selectively disrupting the celiac plexus without the need to open the abdominal cavity and handle the stomach and intestines would help address this surgical phenomenon.

In conclusion, the early development of hypertension in DOCA-salt treated animals can be established by either a redistribution of blood volume and by an increase in total blood volume, and is based on if surgical manipulation is involved. Overall, a decrease in splanchnic blood volume is not *required* for the development of DOCA-salt hypertension in this study

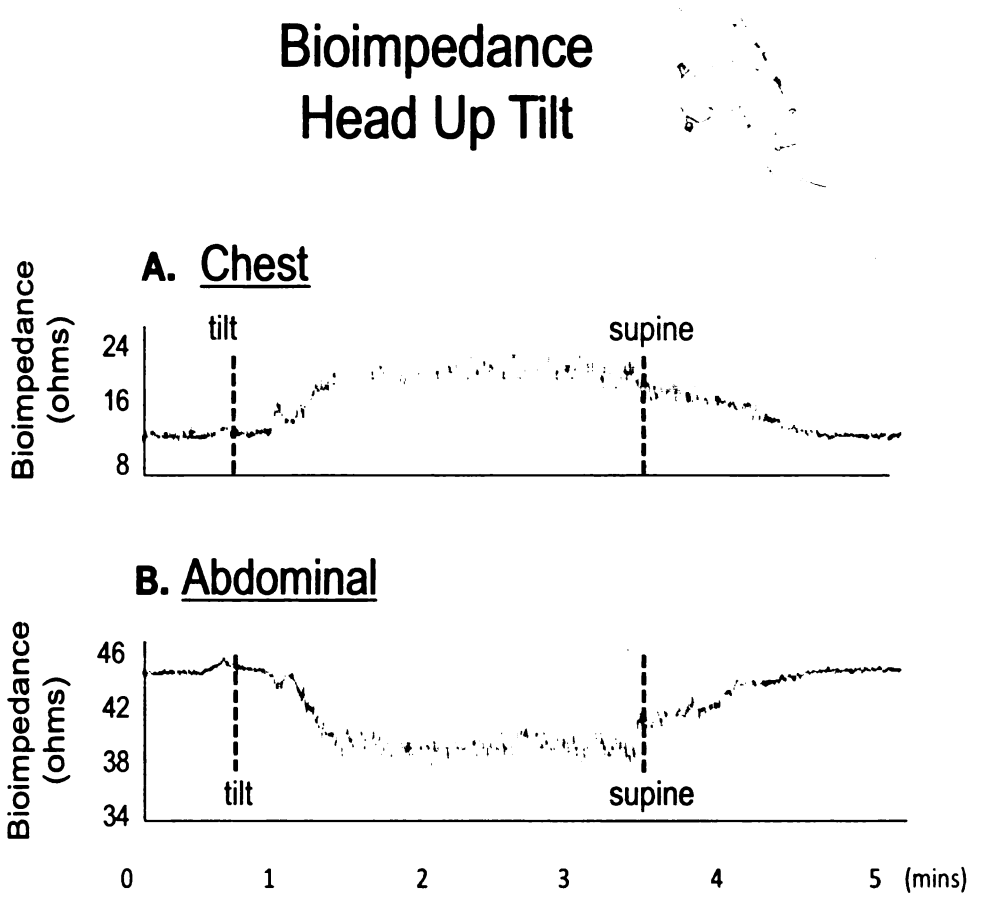

Figure 3: Chest and abdominal bioimpedance recording during a head up postural tilt. The animal began in a supine position and was brought to a ~90° HUT and finished in a supine position. The duration of the experiment was 5 minutes.

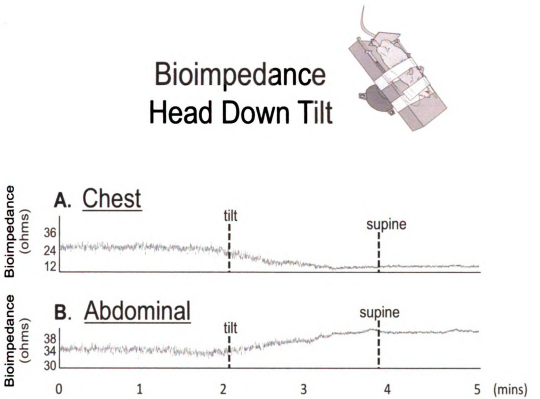

Figure 4: Chest and abdominal bioimpedance recording during a head down postural tilt. The animal began in a supine position and was brought to ~90° HDT and finished in a supine position. The duration of the experiment was 5 minutes.

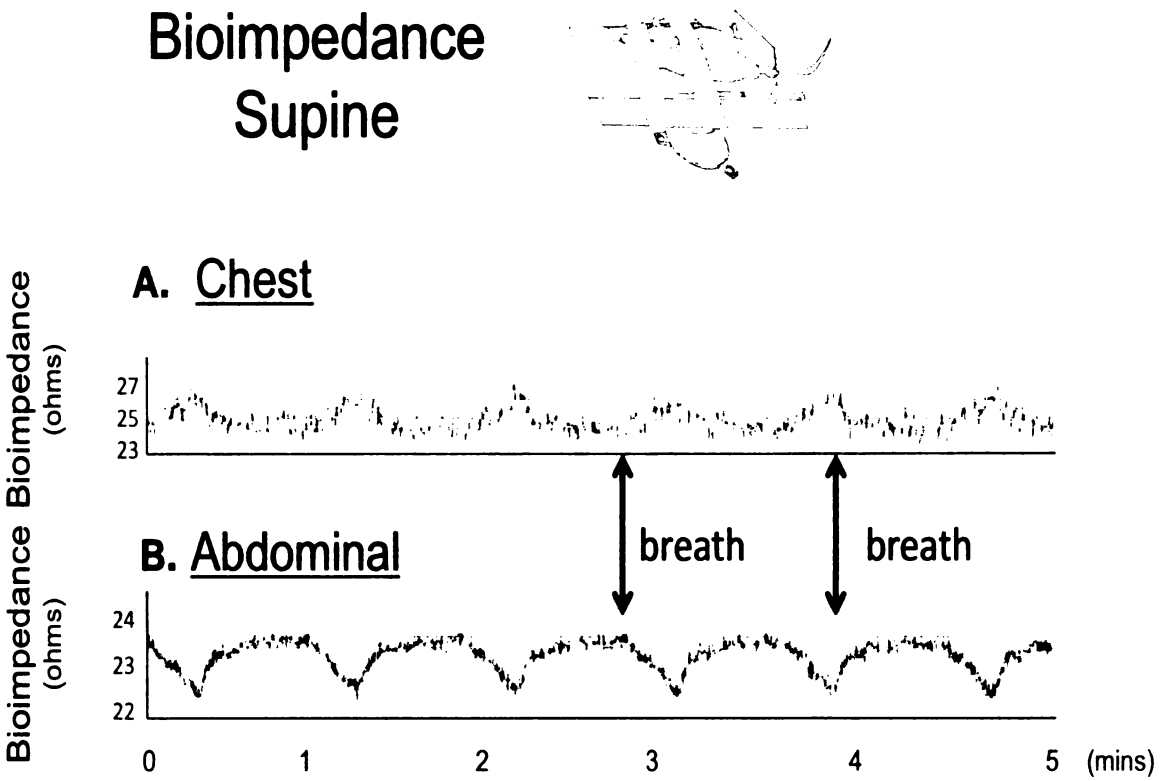

Figure 5: Bioimpedance recordings during a supine position. The duration of the experiment was 5 minutes.

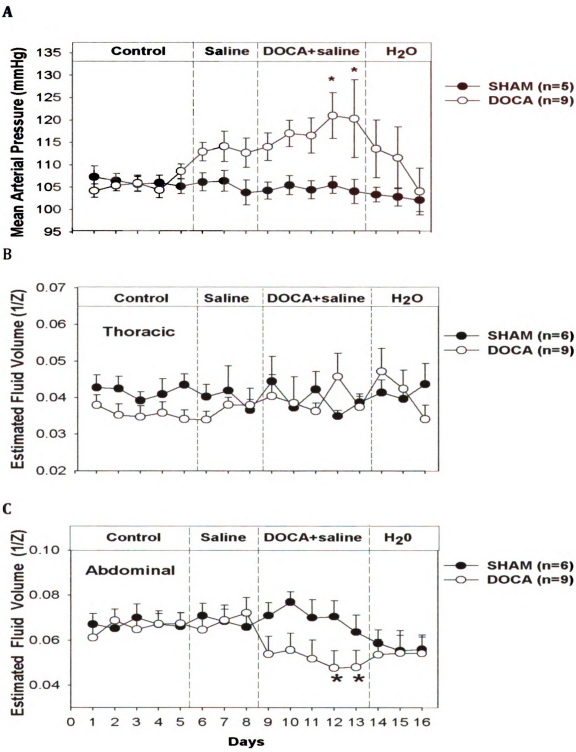

Figure 6: Mean arterial pressure (**A**), estimated thoracic fluid volume (**B**) and estimated abdominal fluid volume (**C**) response in rats treated with DOCA-salt or distilled water. * Significant difference ($p<0.05$) compared to control day 2.

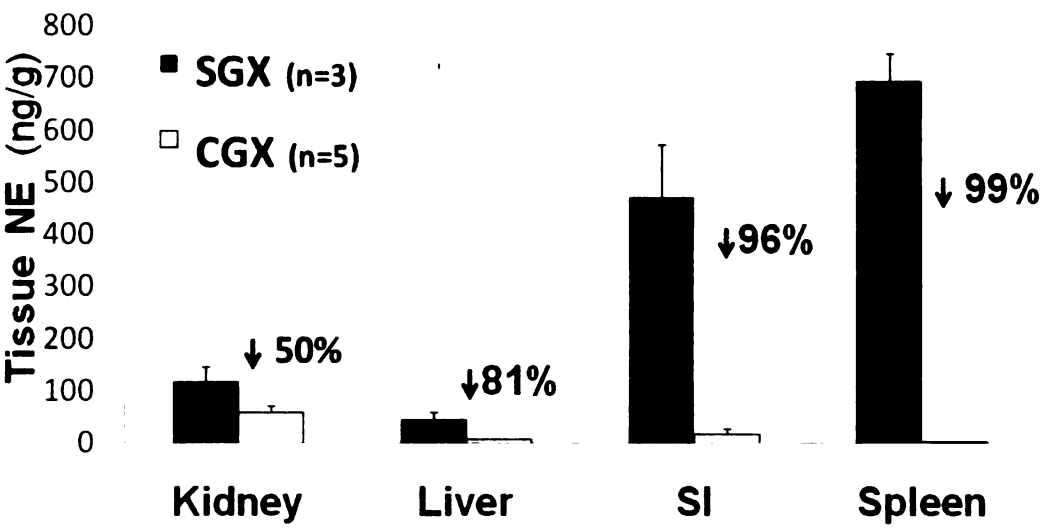

Figure 7: Tissue norepinephrine (NE) content in the splanchnic organs in response to celiac ganglionectomy (CGX) and SHAM-operated (SGX) in DOCA-salt treated rats.

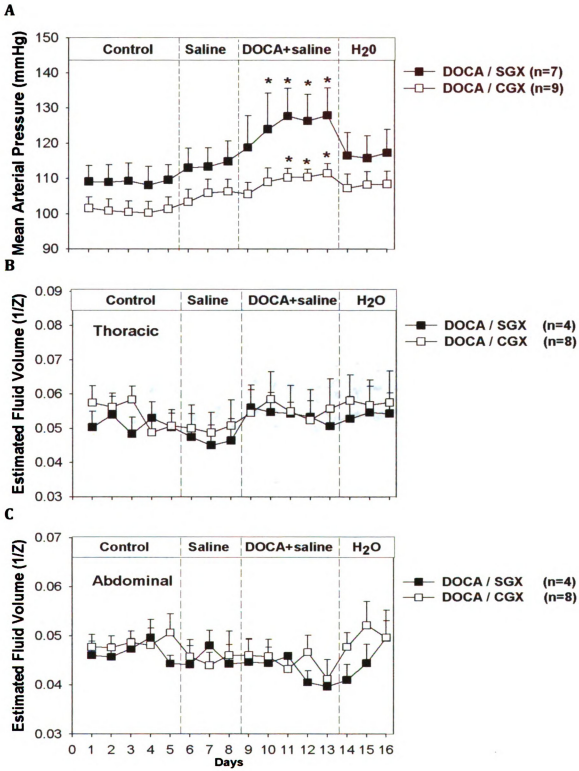

Figure 8: Mean arterial pressure (**A**), estimated thoracic fluid volume (**B**) and estimated abdominal fluid volume (**C**) response to DOCA-salt in celiac ganglionectomized (CGX) or SHAM operated rats

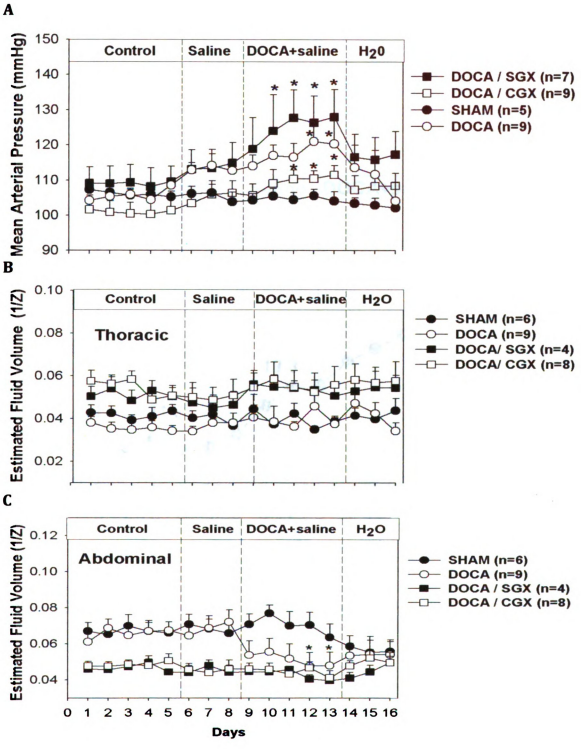

Figure 9: Mean arterial pressure **(A)** and the response of estimated thoracic **(B)** and abdominal **(C)** fluid volume in abdominal surgery rats (CGX and SGX) and non-abdominal surgery rats (DOCA and SHAM).

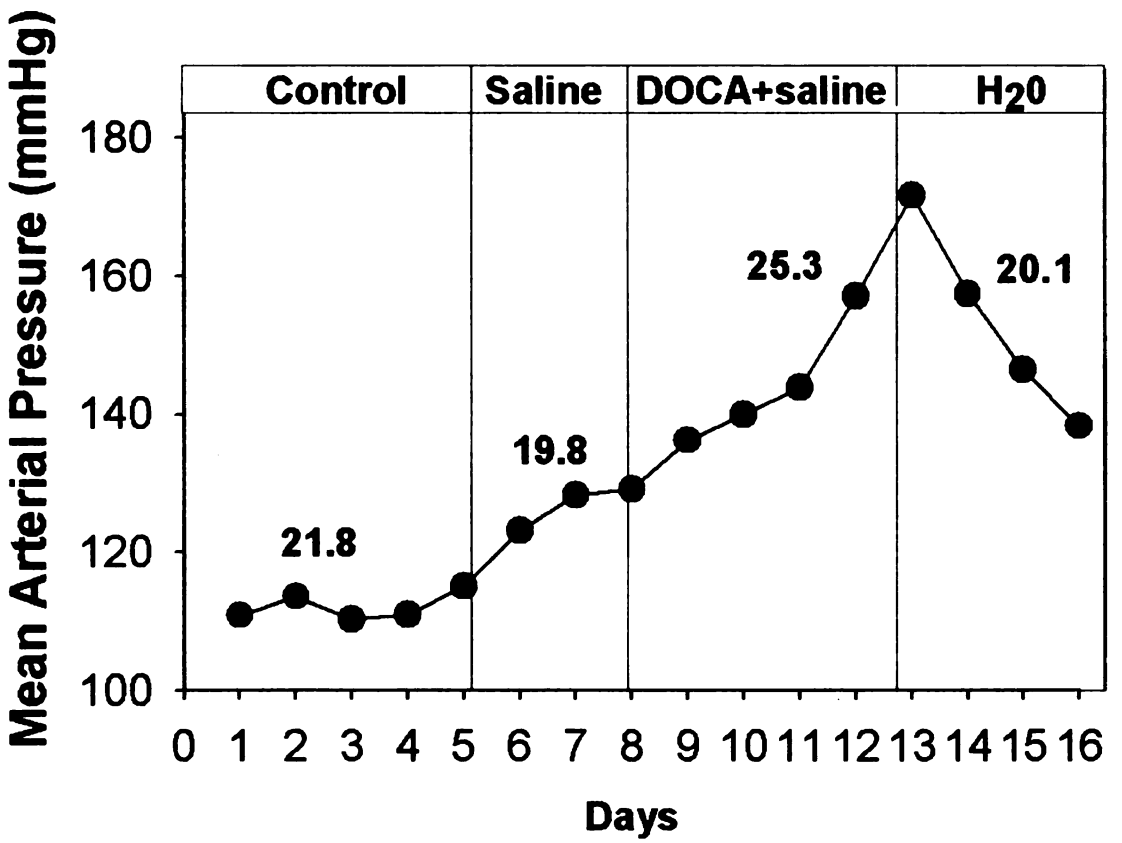

Figure 10: Mean arterial pressure response to DOCA-salt in SHAM operated (SGX) rat (n=1). The numbers in the box represent plasma volume (ml).

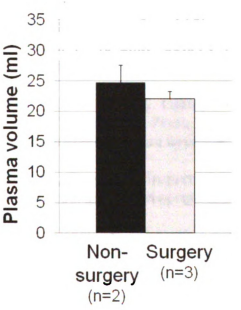

Figure 11: The plasma volume results for non-abdominal and abdominal surgery rats.

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